Design, Synthesis, Characterization and Antioxidant Evaluation of Novel Phenolic Derivatives

A THESIS SUBMITTED TO

Institute of Advanced Research

For The Award of the Degree of

DOCTOR OF PHILOSOPHY
In
CHEMISTRY

By

Ms. Chandani G Halpani
(Reg. No. UIAR/ 11382)

UNDER THE GUIDANCE OF
DR. SATYENDRA MISHRA

Department of Biotechnology and Bioengineering
Institute of Advanced Research, Gandhinagar-382426, Gujarat-India

February 2023

SYNOPSIS

The thesis, *"Design, Synthesis, Characterization and Antioxidant Evaluation of Novel Phenolic Derivatives,"* focuses on the design, synthesis and characterization of chalcones, stilbenes, curcumin and cinnamic acids, and study their structure and antioxidant activity. The work presented in the thesis has been divided into five chapters and a brief description is given below.

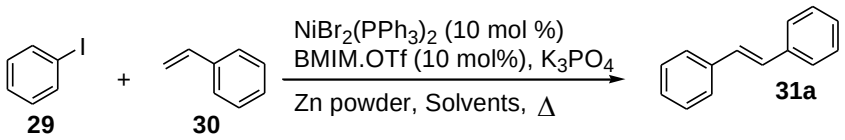
Chapter 1: Introduction

In Chapter 1, a thorough review of the literature on polyphenolic compounds is presented, with a particular emphasis on chalcones, stilbene, curcumin, and derivatives of cinnamic acid. Antioxidants are of great interest to researchers due to their hidden role in biological system protection. Although all living organisms have their own antioxidant defense system, no one can deny the importance of dietary or exogenous antioxidants in disease prevention. As a result, there is a need to discover new and effective free radical scavengers from natural sources that have few or no side effects for use in preventive and/or therapeutic medicine. Natural antioxidant molecules can be synthesized in the laboratory into a variety of more effective derivatives to study their biological activity. Many studies have shown that polyphenols, in addition to free radical scavenging, play an important role in the inhibition of hydrolytic and oxidative enzymes, anti-inflammatory effects, and a variety of other biological or pharmacological activities.

Chapter 2: Design and Development of Novel Synthetic Method for the Synthesis Stilbene and Cinnamate Derivatives

In Chapter 2, a simple and straightforward procedure for the Heck C-C coupling reaction of various aryl halides and olefins catalyzed by nickel is demonstrated. The reaction was catalyzed by a combination of $NiBr_2$ $(PPh_3)_2$, zinc powder, additive (BMIM.OTf), and K_3PO_4 acting as a base in the presence of solvents N,N-dimethylacetamide (DMA) and tetra-n-butylammonium bromide (TBAB). The arylation of methyl acrylate and styrene with various aryl iodides under optimized catalytic systems yielded high product yields. It is worth noting that bases (K_3PO_4), additives (BMIM.OTf), and solvents (TBAB/DMA) all have a significant impact on reaction efficiency and have been identified as the most favorable conditions.

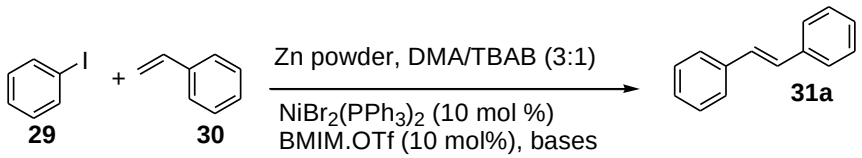
Table 2.1: Optimization of the solvent



Entry	Solvent	Base	Additive	Temp°C	Time (h)	Yield[a]
1	DMF	K_3PO_4	BMIM.OTf	120	10	35
2	DMSO	K_3PO_4	BMIM.OTf	120	8	25
3	DMA	K_3PO_4	BMIM.OTf	120	8	50
4	NMP	K_3PO_4	BMIM.OTf	120	8	40
5	CH_3CN	K_3PO_4	BMIM.OTf	80	12	35
6	1,4-Dioxane	K_3PO_4	BMIM.OTf	100	12	25
7	TBAB	K_3PO_4	BMIM.OTf	120	16	35
8	DMA+TBAB	K_3PO_4	BMIM.OTf	120	4	75

Next, the reaction was optimized for the effective base, and it was found that K_3PO_4 is the best among the tested bases Na_2CO_3, K_2CO_3 and Cs_2CO_3, so it has been used in this study.

Table 2.2: Optimization of the base



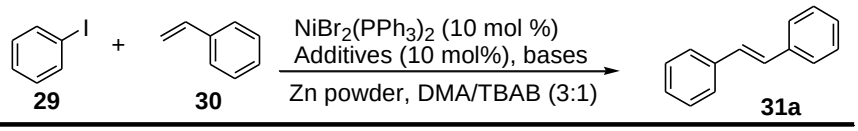
Entry	Solvent	Base	Additive	Temp°C	Time (h)	Yield[a]
9	DMA	Na_2CO_3	BMIM.OTf	120	16	35
10	DMA	Cs_2CO_3	BMIM.OTf	120	8	30
11	DMA	K_3PO_4	BMIM.OTf	120	8	50
12	DMA	K_3PO_4	BMIM.OTf	120	8	55

The effects of solvents, bases, additives, mol% of nickel complex, and halide type on the Heck reaction were investigated in order to determine the optimal reaction conditions. In order to optimize the reaction conditions, the influences of solvents, bases, additives, mole% of nickel complexes and halide types were investigated. Various solvents and DMF, DMSO, DMA, NMP, CH_3CN, 1,4-dioxane, and tetra-n-butyl

ammonium bromide (TBAB) were studied under similar conditions. But got excellent results with DMA.

Additive BMIM.OTf was found to be the best in terms of the yields and reaction time.

Table 2.3: Optimization of the of additive



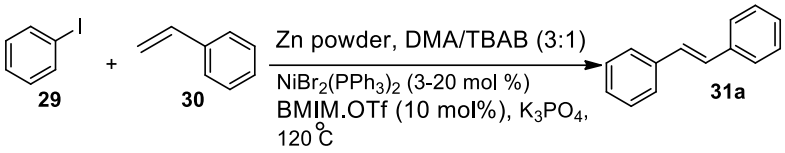
29 **30** **31a**

Entry	Solvent	Base	Additives	Temp°C	Time (h)	Yield[a]
13	DMA+ TBAB	K_3PO_4	BMIM.OTf	120	4	75
14	DMA+ TBAB	K_3PO_4	TBS-OTf	120	6	35
15	DMA+ TBAB	K_3PO_4	TMSOTf	120	6	40

Screening of Loading of catalyst:

The catalyst loading percentage is optimised after the solvent system and additives have been optimised (**Table 2.4**).

Table 2.4 Optimization of catalyst (mol %) for Heck reaction of Iodobenzene and Styrene

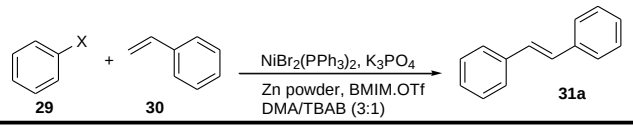


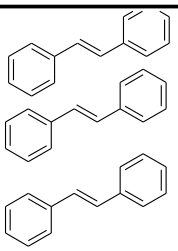
29 **30** **31a**

Entry	Catalyst (mol %)	Time(h)	Yield %
1	20	6	76
2	15	6	74
3	10	6	72
4	5	6	74
5	3	6	60

Results of nickel catalyzed the Heck reaction between aryl halide and styrene clearly shows that the product yield is exaggerated by the halogen species. Aryl iodide gives the product a higher yield than other halides.

Table 2.5: Optimization of the aryl halide in nickel –catalyzed Heck reactions



Entry	ArX	Product	Yield[b]
1			75
2			60
3			50

Screening Substrate Scope under optimized condition:

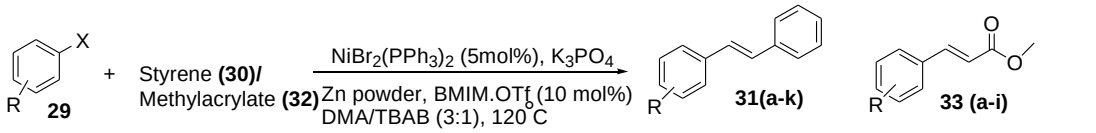
This chapter discusses the use of nickel metal complex catalysts in the Heck reaction. The effects of various solvents, additives, and bases on activity and selectivity were also investigated. In the current study, a novel route to synthesize stilbene and acrylate derivatives has been proposed using a combination of $NiBr_2$ $(PPh_3)_2$, zinc powder, additive (BMIM.OTf), and K_3PO_4 as a base in the presence of solvents N, N-dimethylacetamide (DMA), and tetra-n-butyl ammonium bromide (TBAB).Heck products (stilbenes) were synthesized by reacting halo aryl compounds with terminal olefins (styrene/methyl acrylate) at 120 °C in the presence of K_3PO_4, $NiBr_2$ (PPh_3), BMIM.OTf (additive), zinc powder, and the solvent DMA/TBAB (3:1). The crude desired product was purified using silica gel column chromatography with ethyl acetate/hexane as the eluent, yielding the pure corresponding compound with an isolated yield of 54-96%.The structural characterizations of the synthesized compound were established by recording their ^{1}H NMR and ^{13}C NMR as shown in experimental data and spectra.

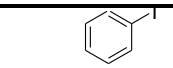
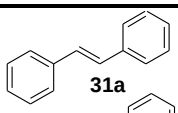
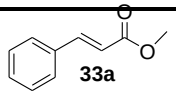
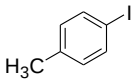
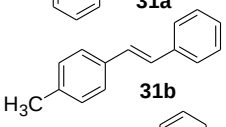
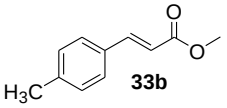
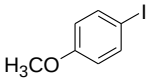
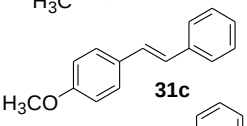
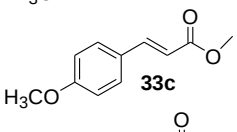
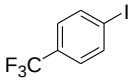
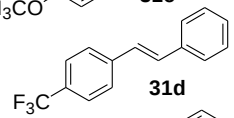
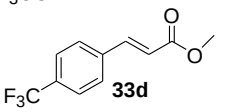
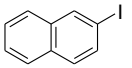
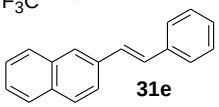
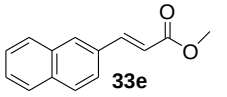
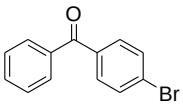
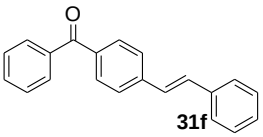
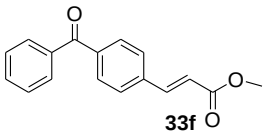
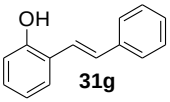
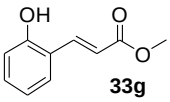
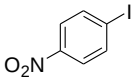
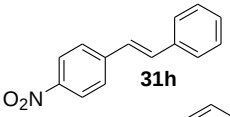
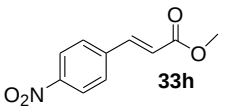
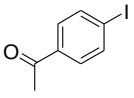
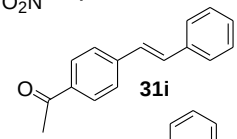
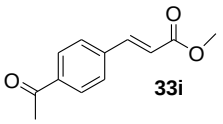
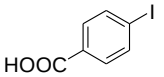
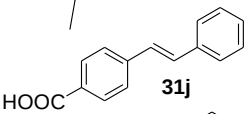
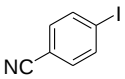
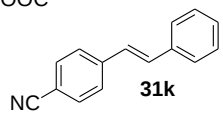
Under the optimized reaction conditions a wide range of aryl halides bearing electron-donating and electron-withdrawing groups coupled with aryl/alkyl olefins, affording the heck products in moderate to good yields and results are summarized in **Table 2.6**. It was found that in the presence of the new catalytic system, C-C coupling occurred under mild conditions, and the corresponding cross-coupling products were obtained in good to excellent yields. It seems that aryl halides with electron-withdrawing groups tend to provide the best result yields

Aryl iodides were discovered to be more reactive than bromides and chlorides, whereas styrene derivatives were discovered to be more reactive than acrylate derivatives. In general, aryl halides with strong electron-withdrawing substituent's (i.e., more -I or -R

effect) yielded more, whereas those with stronger electron-donating substituent's (i.e., more +I or +R effect) yielded less.

Table 2.6 Substrate scope for nickel-Catalyzed Heck Reactions of Aryl halides with styrene/ methyl acrylate (Terminal Olefins)



Entry	ArX	Product (**31a-31**k)	Yield [a]	Entry	Product (**33a-33ai**)	Yield[a]
1		 **31a**	75	12	 **33a**	52
2		 **31b**	54	13	 **33b**	35
3		 **31c**	60	14	 **33c**	45
4		 **31d**	95	15	 **33d**	85
5		 **31e**	75	16	 **33e**	55
6		 **31f**	85	17	 **33f**	72
7		 **31g**	65	18	 **33g**	75
8		 **31h**	96	19	 **33h**	89
9		 **31i**	75	20	 **33i**	80
10		 **31j**	82			
11		 **31k**	85			

Reaction conditions: Iodobenzene (1 mmol), styrene (1.2 mmol), K_3PO_4 (2.5 mmol), $NiBr_2(PPh_3)_2$ (5 mol %), additive (BMIM.OTf; 10 mol %), DMA/TBAB (3:1), zinc powder (1.1 mmol), temperature (120°C), 4h; [a]Isolated yield

The DPPH and ABTS techniques were used to assess the antioxidant activity of each molecule (**Table 2.8**) and L-ascorbic acid was used as control. The results are displayed as IC_{50} values. The table unequivocally demonstrates that most of the synthetic substances have a poor antioxidant activity. The compound **31g** and **33i** showed superior free radical scavenging activity than L-ascorbic acid in the ABTS and DPPH experiment. The results imply that the chemical **31g** and **33i** might be a viable antioxidant molecule.

Chapter 3: Calcium Catalyzed Claisen Schmidt Reaction: Synthesis of Chalcone Derivatives

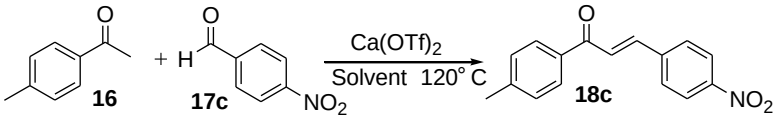
Chapter 3 describes a simple and straightforward procedure for the chalcone in the presence of $Ca(OTf)_2$ and NBu_4. Chalcone is a biphenyl compound with two aryl moieties connected by -α, β-unsaturated ketone and a good Michael acceptor and undergoes nucleophilic addition. Chalcone is a secondary metabolite that has attracted the interest of researchers because of its possible medicinal value. Typically, under usual acidic or basic circumstances, aromatic ketones and aldehydes combine to produce chalcone and its derivatives. This process is frequently known as Claisen-Schmidt condensation. Chalcone and its derivatives have undergone extensive synthetic attempts during the last few decades.

However, in most cases of these transformations, strong bases, noble metal catalysts, ligands, and harsh operations are always required. Due to their great natural abundance, affordability, and safety, alkaline earth metal catalysts have recently been recognized as alternatives to transition metal and lanthanide-based catalysts. In organic synthesis, the Claisen-Schmidt condensation process is frequently employed, from the production of biologically active small molecules to the total synthesis of more complex natural products. In the literature, there are numerous instances of its application.

We have studied many factors that may affect the reaction, including reaction time, reaction solvent, molar percentage of catalyst and molar percentage of additives. In order to carefully examine the effect of additives on the reaction, we check the amount of additives and the reaction of the catalyst $Ca(OTf)_2$. Further, in order to find out the appropriate catalyst $Ca(OTf)_2$ loading, a model reaction was performed employing 4-nitrobenzaldehyde and 4-methyl acetophenone in the presence of 4 mol%, 5 mol %, 10 mol % and 15 mol % of catalyst without any solvent (**Table 3.1**, entries 6–10). It was discovered that using 5% of the catalyst produced the highest yield in the shortest amount

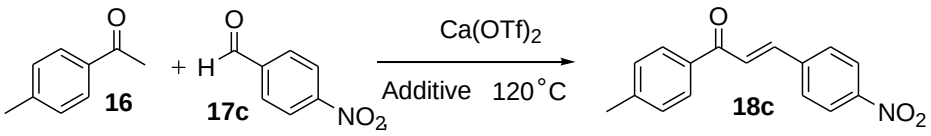
of time. The yield was neither improved nor decreased when the catalyst was loaded at higher percentages (10 to 15 mol %). The yield was drop when the catalyst concentration is decreased from 5 mol% to 4 mol%, hence 5 mol% is the effective amount that must be present for the best yield.

Table 3.1: Optimization of the solvents and Catalyst



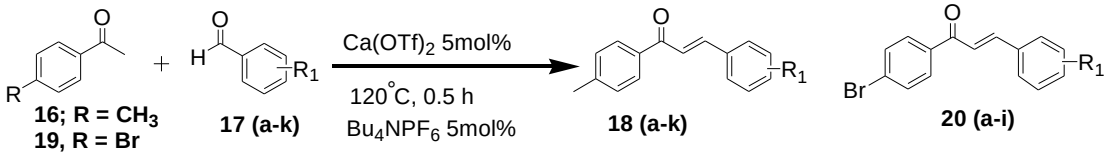
Entry	$[Ca(OTf)_2]$ (mol%)	Solvents	Time (h)	Temp(°C)	Yield
1	10	H_2O	6	100	25
2	10	CH_3CN	8	82	15
3	10	Toluene	8	110	NR
4	10	EtOH	8	78	NR
5	10	$CHCl_3$	8	62	NR
6	10	Neat	6	120	56
7	15	Neat	6	120	55
8	5	Neat	6	120	55
9	4	Neat	6	120	52
10	6	Neat	6	120	55

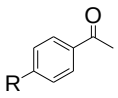
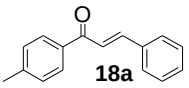
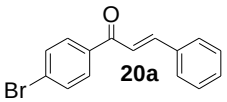
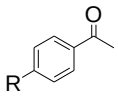
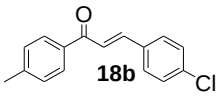
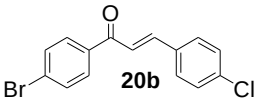
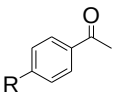
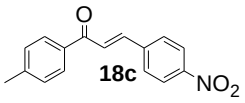
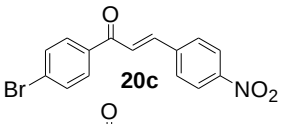
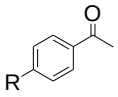
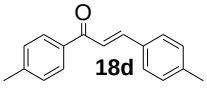
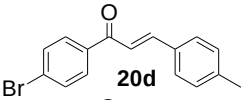
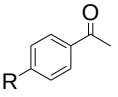
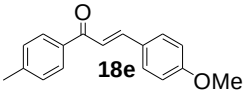
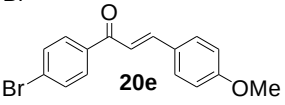
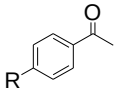
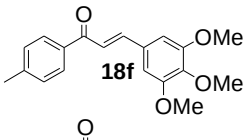
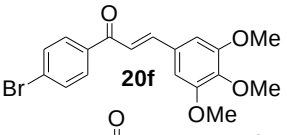
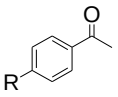
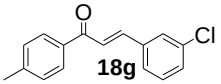
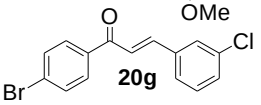
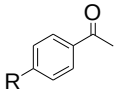
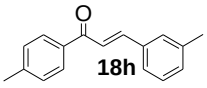
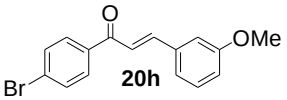
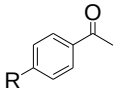
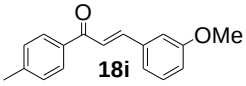
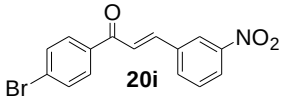
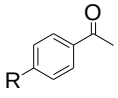
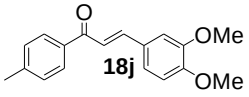
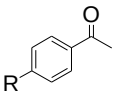
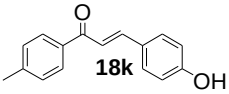
Table 3.2: Optimization of the loading of additive (mol %)



Entry	$[Ca(OTf)_2]$ (mol%)	Additive (mol%)	Solvents	Time (h)	Temp(°C)	Yield[a]
1	5	$NaPF_6$ (5%)	Neat	6	120	52
2	5	NaOTs (5%)	Neat	6	120	55
3	5	Py.OTs (5%)	Neat	6	120	50
4	5	Proline (5%)	Neat	6	120	45
5*	5	Bu_4NPF_6 (5%)	Neat	0.5	120	96
6	5	Bu_4NPF_6 (10%)	Neat	0.5	120	90
7	5	Bu_4NPF_6 (15%)	Neat	0.5	120	90
8	0	Bu_4NPF_6 (5%)	Neat	8	120	trace
9	0	Bu_4NPF_6 (10%)	Neat	8	120	trace
10	0	Bu_4NPF_6 (15%)	Neat	8	120	trace

Table 3.3: Substrate scope in the Ca(II) catalyzed Claisen Schmidt reaction of 4-Methyl acetophenone/4-Bromo acetophenone with substituted aromatic aldehyde



Entry	1	R_1	Product (3a-3k)	Yield[a]	Product (3a-3k)	Yield[a]
1		H	 **18a**	80	 **20a**	85
2		4-Cl	 **18b**	94	 **20b**	95
3		4-NO_2	 **18c**	98	 **20c**	99
4		4-CH_3	 **18d**	88	 **20d**	80
5		4-OCH_3	 **18e**	92	 **20e**	88
6		3, 4, 5-OCH_3	 **18f**	96	 **20f**	96
7		3-Cl	 **18g**	94	 **20g**	97
8		3-Me	 **18h**	85	 **20h**	85
9		3-OCH3	 **18i**	93	 **20i**	98
10		3,4-OCH3	 **18j**	95		
11		4-OH	 **18k**	92		

Primarily, in order to explore the task of additive on reaction, we scrutinized the reaction with seven different additives such as $NaPF_6$, $NaBF_4$, Bu_4NPF_6, NaOTs, $NaNO_3$, CF_3SO_2Na, proline along with catalyst $Ca(OTf)_2$. Among all these additives, Bu_4NPF_6 provided excellent results. It can be concluded that in order to determine the actual

performance of the catalyst $Ca(OTf)_2$, addition of the additive (Bu_4NPF_6) is indispensable in reaction (**Table 3.2**; entry 5).

Bearing in mind the significance of the additive Bu_4NPF_6 we also performed the reaction in the presence of 5 mol% of Bu_4NPF_6 and absence of catalyst, $Ca(OTf)_2$ and it was observed that no product formation occurs in the absence of catalyst $Ca(OTf)_2$ even after 8 h (**Table 3.2**; entry 8). Additive only helps in augmentation of catalytic activity of catalysts. Interestingly, no product formation was observed in the absence of catalyst $Ca(OTf)_2$ (**Table 3.2**; entries 8-10). Therefore, in order to determine the actual effectiveness of the catalyst $Ca(OTf)_2$, an additive (Bu_4NPF_6) must be added in reaction (**Table 3.2**; entry 5).

With this optimal reaction condition, we extended our research with different substituted acetophenone and substituted benzaldehydes as reaction examples (**Tables 3.3**). The substituted acetophenone and benzaldehyde were coupled in situ to obtain the corresponding chalcone with excellent yield, and it was characterized by ^1H NMR and ^{13}C NMR. The method has broad substrate tolerance and mild operating conditions, and can produce various chalcone derivatives in medium to high yields from readily available starting materials.

Compound **18k** is the most active amongst all the tested compounds (DPPH IC_{50} 65±2.03 µM, ABTS IC_{50} 4±1.2 µM) and it demonstrated two fold enhanced antioxidant activity than ascorbic acid (DPPH IC_{50} 118±2.8 µM, ABTS IC_{50} 7.8±0.8 µM). The phenolic group in compound **18k** may be responsible for the DPPH radical possible absorption. However, there are additional aspects than chemical structure, such as reactants like Michael acceptors, the capacity to chelate transition metals, solubility, and interactions with biological membranes.

There is no report on the synthesis of chalcone in the presence of the $Ca(OTf)_2$-$NBu_4.BF_4$ catalyst system to our knowledge. Here, we report the use of a Lewis acid-based calcium catalyst system to synthesize many aromatic chalcones under solvent-free, alkali-free, mild operation and broad substrate tolerance conditions to provide moderate to good yields Corresponding products. In DPPH and ABTS assays, the synthesised chalcones derivatives (**18k**) performed better as antioxidants than ascorbic acid.

Chapter 4: Design and Synthesis of Benzyledine Derivatives of Curcumin and Evaluation of their Antioxidant Activity

Curcumin is the main curcuminoids of turmeric and is isolated from the root of the turmeric plant. Curcumin has been extensively researched due to its wide range of medicinal properties, including its anti-cancer, antioxidant, antibacterial, and anti-angiogenic activities, anti-inflammatory and anti-HIV, anti-arthritis, anti-depressive, anti-aging, anti-malarial, antibacterial, and anti-diabetic activities. The activity and instability of curcumin in aqueous solutions have been attributed to the presence of phenolic groups in the compound. α, β- Unsaturated dicarbonyl moiety and keto-enol tautomerism in curcumin are to responsible for some of its physical and metabolic instability, which necessitates its modification.

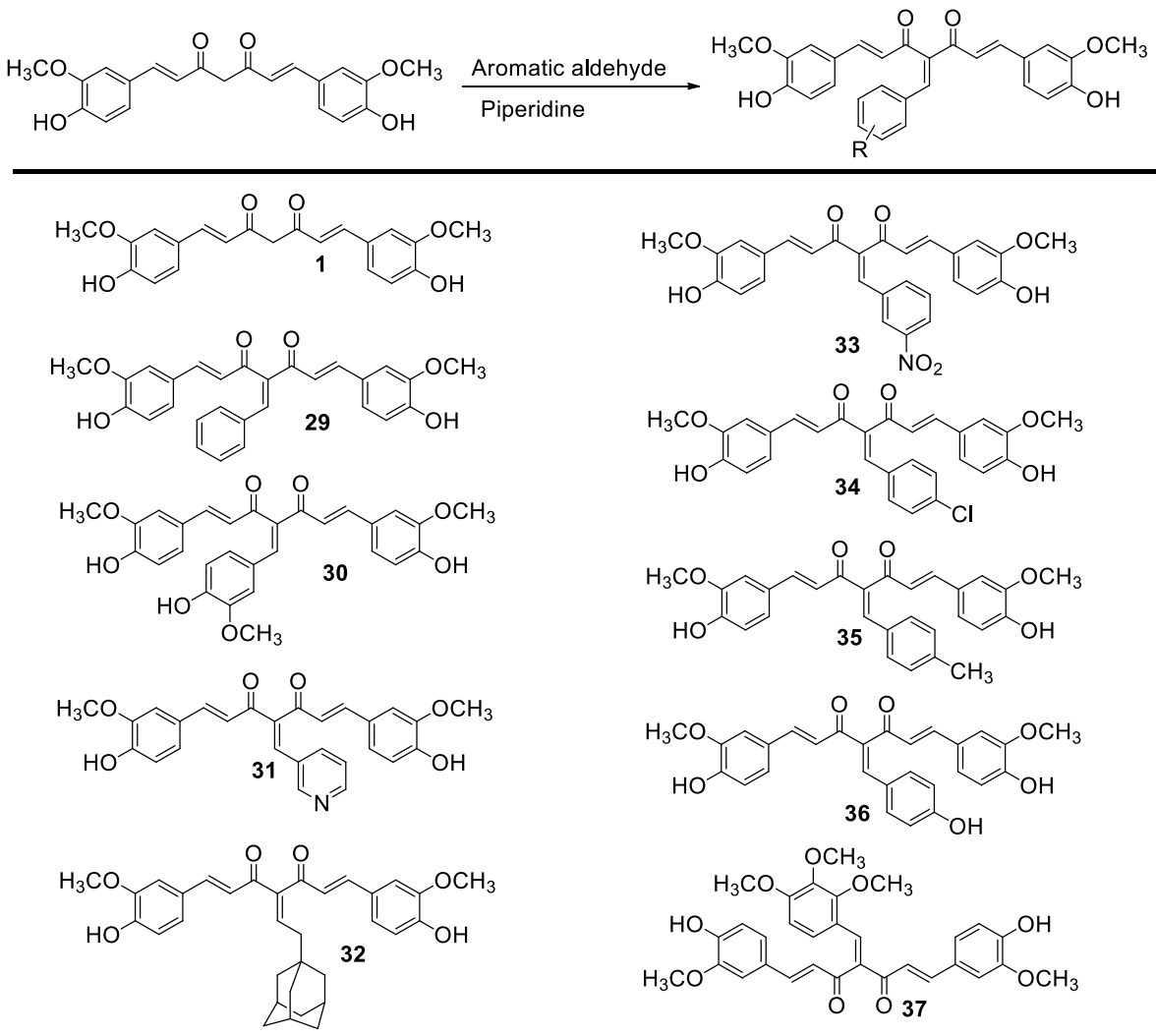


Figure 4.4: Synthesis of curcumin benzyledine analogs

To achieve this goal, we developed a series of curcumin Knoevenagel condensates and tested their antioxidant activity against unmodified curcumin treatment. The effect of curcumin's substituted Knoevenagel condensate on antioxidants was investigated in this study. The synthesis and identification of new 4-arylidene curcumin analogues (Knoevenagel condensates of 1,3-diketone curcumin analogues with aromatic aldehydes) as a new class of potential antioxidant agents are described in this study.

We designed and synthesized new Knoevenagel condensates of curcumin and evaluated their antioxidant activity. A little amount of methanol was used to dissolve the curcumin, and the appropriate amount of aromatic aldehyde (in a stoichiometric ratio) was then slowly added while stirring (**Figure 4.4**).

Antioxidant Activity

The free radical scavenging capacity of all compounds was determined using the DPPH and ABTS assays. The IC_{50} value denotes the sample concentration capable of scavenging 50% of the free radicals. A low IC_{50} value indicates that the sample compound has high antioxidant activity.

DPPH assay:

The DPPH assay is shown in **Table 4.** due to the presence of electron donating groups as well as hydroxyl groups at the benzyledine moiety, compounds **30, 36,** and **37** demonstrated significantly better DPPH radical scavenging ability (IC_{50} = 35±2.02 μM, 42±0.9 μM and 58±2.0 μM, respectively) than curcumin (IC_{50} = 75±1.45). The ability of these compounds to donate electrons to the DPPH radical, resulting in the formation of $DPPH_2$ neutral While electron withdrawing group bearing compounds had the lowest radical scavenging power **33** (p-NO_2 containing curcumin knovengeal condensate; IC_{50} = 116±2.02 μM), **34** (p-chloro containing curcumin knovengeal condensate; IC_{50} = 112±1.45 μM) ($p<0.05$) and no significant ($p>0.05$) difference was found for ascorbic acid

ABST assay:

All compounds have IC_{50} values between 1.42 μM and 13.79 μM. Due to the presence of an electron donating group, it was discovered that compounds **30, 36**, and **37** had higher activity and were substantially different ($p<0.05$) from curcumin and ($p<0.01$) from ascorbic acid. Improved electron donation to free radical ABTS leads to increased antioxidant capacity. However, there were no appreciable differences ($p>0.05$) in

compound **35** capacity to scavenging ABTS cation radicals. Compounds **29, 31, 32, 33** and **34** inhibited the least amount of free radicals, with IC50 values of 13.79±0.04 µM, 7.56±0.03µM, 12.44±0.25µM, 11±0.075µM, and 8.3±0.089µM respectively

Table 4.1: Antioxidant activity of synthesized conjugates (**29-37**), curcumin, ascorbic using DPPH and ABTS assay

Compound code	IC_{50} (µM)±SD	
	DPPH	ABTS
29	98±1.45	13.79±0.04
30	35±2.02	1.42±0.11
31	93±2.02	7.56±0.03
32	108±1.73	12.44±0.25
33	116±2.02	11±0.075
34	112±1.45	8.3±0.089
35	88±1.15	3.5±0.28
36	42±0.9	1.73±0.026
37	58±2.0	2.53±0.145
Ascorbic acid	111±120	6.4±0.01
Curcumin(1)	75±1.45	3±0.036

Fe^{2+} Chelating activity:

In this assay, curcumin interfered with the development of the ferrous–ferrozine complex, indicative of that curcumin has chelating activity and is competent to capture ferrous ion with a higher binding affinity than ferrozine.

In addition to the DPPH and ABTS free radical scavenging activities, the ferrozine-based iron assay was used to determine the Fe^{2+} chelating activity of all the synthesised compounds. **Table 4.3** shows the Fe^{2+} ion-chelating activity of all synthetic curcumin derivatives, EDTA, curcumin, at 60 µM and 30 µM concentrations. Compounds **30, 31, 36** and **37** demonstrated greater Fe^{2+} chelation capacity than curcumin and EDTA at both concentrations. At lower concentrations, all compounds found better than curcumin .while at 30 µM, compound **30, 36, 37** exhibits two fold better chelating capacity than EDTA. As this compounds containing extra C–OH, OCH_3 on benzyledine substitution and C=O functional group may chelate Fe^{2+}metal ions efficiently than curcumin.

Table 4.3: Fe^{2+} ion chelating activity of EDTA, Curcumin, and curcumin analogues (**29-37**) at 60 µm and 30 µm concentration

Compound code	Ferrous ion chelating effect (%) ± SD	
	60µm	30 µm
29	27±2.7	22±1.3
30	54±3.5	43±2.4
31	42±1.8	28±2.7
32	24±1	18±2.9
33	24±0.3	20±0.8
34	29±0.2	15±1.6
35	27±1	15±4
36	56±0.1	49±2.7
37	57±2.4	55±2.7
Curcumin(1)	28±3.7	4±0.2
EDTA	35±2.1	23±1.3

In conclusion, a number of curcumin derivatives containing the benzyledine moiety were effectively synthesised in good yields ranging from 48 to 75%. The structure of synthesised curcumin Knoevenagel condensates was determined using spectroscopy methods. The synthetically produced molecules showed a variety of antioxidant properties that could be very promising. Using the DPPH, ABTS, and Fe^{2+} Chelating activity, the antioxidant capacity of each molecule was evaluated. In the ABTS method, compounds **30, 36,** and **37** have stronger antioxidant activity than, ascorbic acid, and curcumin; however, compound **35** has better activity than only ascorbic acid. Compounds **30, 36,** and **37** had higher antioxidant activity in the DPPH experiment compared to curcumin and ascorbic acid. However, all the synthesized molecules exhibit more antioxidant activity than ascorbic acid.

In the Fe^{2+} Chelating activity, compounds **30, 31, 36 and 37** showed greater chelating activity than EDTA. Compound **30** had higher antioxidant activity in the DPPH and ABTS assays compared to all synthetic analogues, and compound **35** outperformed in the ABTS and Fe^{2+} Chelating activity. Compounds **36** and **37** showed greater antioxidant activity across all the assays. All tested phenolic compounds showed stronger antioxidant activity in the DPPH assay than in the ABTS and Fe^{2+} Chelating activity, which were carried out in aqueous solution. Our findings imply that the newly synthesized curcumin analogues may be effective as antioxidant and/or therapeutic agents.

Chapter 5: Synthesis, Characterization, and the Antioxidant Activity of Ferulic Acid and *p*-Coumaric Acid Amide Derivatives

Ferulic acid and p-Coumaric acid derivatives (**19-27**) were synthesized as shown in **Figure 5.1.** Ferulic acid/p-coumaric acid is coupled with substituted benzyl amine in the presence of a coupling agent to yield the feruloyl and coumaroyl amide derivatives. The structures of all the newly synthesized compounds were confirmed by the, ^{1}H NMR, ^{13}C NMR which were in full agreement with their structures

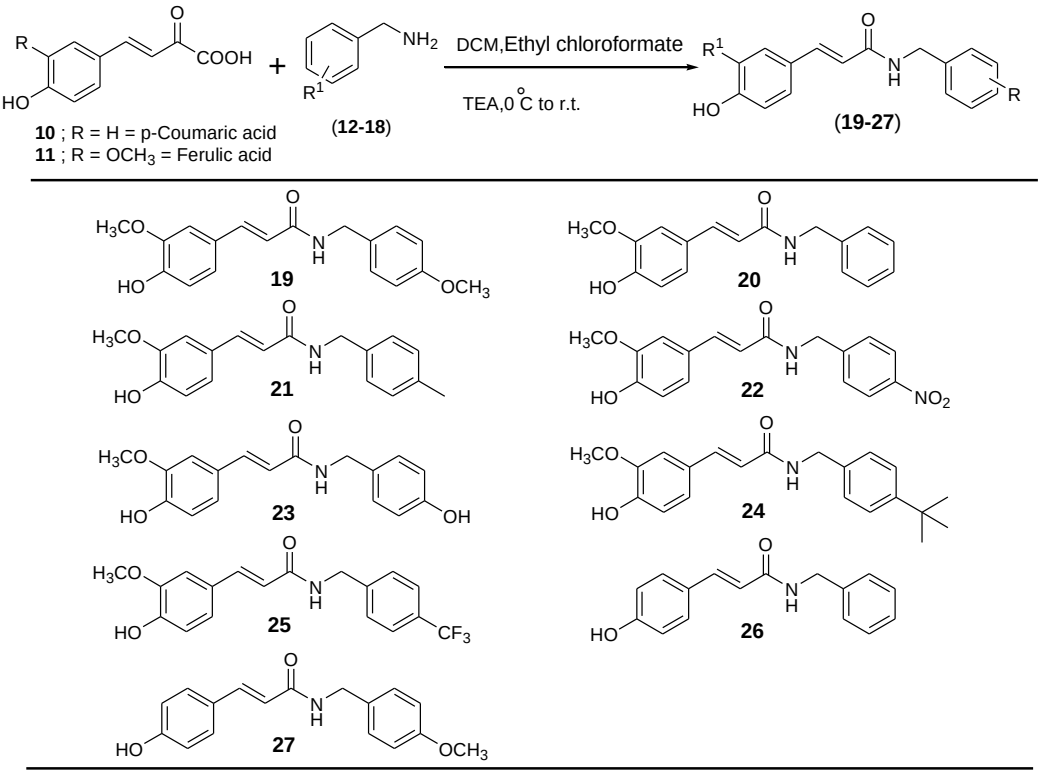


Figure 5.1: Synthesis of Cinnamoyl (Feruloyl/Coumaroyl) Amide derivative

Antioxidant Activity

All of the amide derivatives investigated in this study was subjected to antioxidant screening using the DPPH and ABTS assays. The antioxidant activity was measured as a percentage of radical inhibition using the gulcine method. The IC_{50} value is the sample concentration that can scavenge 50% of the free radicals in the reaction mixture. A low IC_{50} value indicates that the sample compound has high antioxidant activity.

Table 5.1: Antioxidant activity of Cinnamoyl (Feruloyl/p-Coumaroyl) Amide derivative

FA /p-CA Amide	DPPH (IC50)µM±(SD)	ABTS (IC50)µM±(SD)
19	53±3.6	7±1.8
20	58±1.3	10±1.3
21	57±2.5	10±2.5
22	186±1.2	49±0.7
23	29±1.5	5±0.7
24	56±4.3	9±3.2
25	156±0.5	30±1.0
26	84±0.8	7±2.3
27	81±2.1	8±4.3
Ferulic acid	66±2.3	12±2.3
p-coumaric acid	89±1.23	16±0.4
Ascorbic acid	120±3.3	9±1.0

Values are means ± SD of three different determinations

All of the synthesised compounds' free radical scavenging capacity was determined using two free-radical assays, DPPH and ABTS, and the results are shown in **Table 5.1**. Ascorbic acid was used as a standard antioxidant. Antioxidant tests were carried out using synthetic conjugates and starting substrates at various concentrations (200, 100, 50, 25, 12.5, and 6.25 µM). The experiments were repeated three times in order to reproduce the obtained results and statistical analyses In the DPPH assay, conjugates **19, 20, 21, 23,** and **24** have higher antioxidant power (IC_{50} 53±3.6, 58±1.3, 57±2.5, 29±1.5, 56±4.3 µM respectively) than parent ferulic acid (IC_{50}56±2.3 µM) and reference ascorbic acid (IC_{50}120±3.3 µM). These are compounds that have electron-donating substitutions (OCH_3, CH_3, t-but) along with OH. Compounds **22** (IC_{50}186±1.2 µM) and **25** (IC_{50}156±0.5 µM) found to possess poor antioxidant activity due to the presence of NO_2 and CF_3 substitutions. Among all synthesized derivatives Compound **23** found to be most active molecules during DPPH assay with IC_{50} value of 29±1.5 µM.

In the ABTS assay, compounds **19**, **20**, **21**, **23,**and **24** demonstrated higher antioxidant activity (7±1.8, 5±0.7,10±1.3, 10±2.5, 9±3.2 µM) than ferulic acid (12±2.3 µM) and ascorbic acid (19±1.0 µM). While electron withdrawing substituted conjugates **22** (49±0.7 µM), **25** (30±1.0 µM) demonstrated lower activity than parent molecules and ascorbic acid. Conjugates **26, 27** have higher DPPH and ABTS radical scavenging activity than parent p-CA and ascorbic acid. As shown in **Table 5.1**. Trends in antioxidant activity were found to be similar in both the DPPH and ABTS assays. Compound **23** found four

fold better DPPH radical scavenging power and two fold better ABTS cation radical scavenging capacity than Ascorbic acid .We also discovered a positive correlation between the antioxidant activity of synthesized amides in DPPH and ABTS Assay.

In vitro antibacterial activity*:*

Synthetic ferulic and coumaric acid conjugates inhibited Gram-positive and Gram-negative bacterial strains differently (**Table 5.2**). Conjugates **19**, **21**, and **24** were found to have good activity against B subtilis and P. aeruginosa. Compounds **19, 21**, and **24** were more effective than ferulic acid, p-coumaric acid, and kanamycin against *B subtilis* and *P. aeruginosa*. Synthesized conjugates **19-27** are not shown better activity against *E.coli* than parent compounds. They are, however, inactive against *B. megaterium*. Furthermore, compounds **19, 21,** and **24** demonstrated the highest antibacterial activity against *B subtilis* and *P. aeruginosa* when compared to all tested compounds, as well as better inhibitory activity than the standard drug kanamycin. Conjugates with electron donating groups and + I effect (**19, 21,** and **24**) demonstrated good antibacterial activity against *B subtilis* and *P. aeruginosa*. Conjugates **20, 22, 23, 25, 26,** and **27** had lower antibacterial activity against *B subtilis* and *P. aeruginosa* than active compounds **19, 21** and **24.** The findings validate the significance of feruloyl and coumaroyl amide derivatives.

Table 5.2: Antibacterial activity (IC_{50}) of feruloyl and coumaroyl amide derivatives against Gram positive (*B. megaterium, B. subtilis*) and Gram negative (*E. coli*, *P. aeruginosa*)

Compound	*B subtilis* (IC50)µM±SD	*P. aeruginosa* (IC50)µM±SD	*E.coli* (IC50)µM±SD	*B. megaterium* (IC50)µM±SD
19	416±3.3	365±2.8	984±4.2	nd
20	490±1.9	700±0.5	1175±2.4	nd
21	336±2.7	465±1.8	785±4.8	nd
22	551±2.8	754±2.6	685±2.4	nd
24	215±1.3	341±3.6	743±1.3	nd
25	624±1.7	792±1.1	710±2.4	nd
26	587±0.8	621±3.2	1261±1.8	nd
27	677±1.1	710±3.3	899±1.8	nd
Ferulic acid	700±3.4	831±2.9	690±2.3	nd
p-Coumaric Acid	652±3.3	790±3.8	630±1.1	nd
Kanamycin	440±1.8	380±2.0	131±1.8	288±1.3

Ferulic acid and *p*-Coumaric acid are an essential natural lead molecule in the design of compounds of biological interest. By structural modification with a variety of aromatic amines, we were able to synthesize a diverse range of amide derivatives of Ferulic acid and p-Coumaric acid. Based on natural compounds, synthetic compounds could serve as a new therapeutic approach in the medical field. Compounds **19**, **20**, **21**, and **24** demonstrated higher antioxidant activity in both DPPH and ABTS assays than ferulic acid and ascorbic acid. Compound **23** with a phenolic group performed excellent than the parent molecules and ascorbic acid. Ccompounds **19, 21,** and **24** demonstrated the highest antibacterial activity against *B subtilis* and *P. aeruginosa* compared to all tested compounds, as well as better inhibitory activity than the standard drug kanamycin.